

Amendments to the Specification

1. Please amend the paragraph starting on page 24, line 1, as follows.

Extracted bacterial surface proteins (~~10 Fg/lane~~) (~~10 µg/lane~~) were electrophoresed on 10% SDS-PAGE. Gels were stained with Coomassie brilliant blue or transferred onto nitrocellulose membrane using Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio-Rad) (Bio-Rad, Hercules, CA). Unbound sites on blots were blocked with 3% casitone. Blots were probed with LF (~~ICN, 5 Fg/ml~~) (ICN, 5 µg/ml) in PBS TWEEN 20 (PBST) containing 0.1% casitone for 6 h at 4°C, followed by four washes with PBST. Procedures for further probing of blots with rabbit anti-bovine LF antibody and HRP-conjugated donkey anti-rabbit IgG antibody were as described previously (Fang and Oliver, 1999). Blots without probing with LF and rabbit anti-bovine LF antibody were included as negative controls.

2. Please amend the paragraph starting on page 25, line 14, as follows.

Thirty ml of PBS (pH 7.4) containing 30 mg of SDS-extracted *S. uberis* surface proteins were loaded into a bovine LF-coupled CNBr-activated SEPHAROSE 4B column. SDS-extracted surface proteins were incubated with shaking for 2 h at 4°C with 7 ml of SEPHAROSE 4B covalently linked to bovine LF (ICN, 21.4% iron saturation and 97.5% protein content). The LF-SEPHAROSE 4B slurry was loaded into a chromatography column (1.25 cm x 9 cm; total volume 70 ml) (Pfizer, New York, NY). The column was subsequently washed with 10 volumes of TBS (50 mM TRIS-HCl (pH 7.4) + 150 mM NaCl containing 0.1% TRITON X 100) to remove nonspecific-binding proteins using a peristaltic pump at a flow rate of 1 ml/min until

absorbance at 280 nm approached zero. The column was eluted with a sodium chloride gradient from 0.1 M to 1 M NaCl in TBS. Fractions (10ml/fraction) were analyzed by absorbance at 280 nm, SDS-PAGE and Western blot ~~using~~ using LF, rabbit anti-bovine LF antibodies and biotinylated LF as probes. Fractions containing SUAM were pooled, dialyzed against PBS and stored at -70°C until use.

3. Please amend the paragraph starting on page 26, line 9, as follows.

Analysis of fractions revealed the presence of a protein in fraction number 14 to 32 eluted at 0.5M NaCl. The molecular mass was estimated to be ~ 112 kDa using GEL SCAN (Corbett Research, Mortlake, NSW, Australia). Results from SDS-PAGE and Western blot analysis indicated that this band had LF-binding affinity. The yield of purified SUAM was 20 Fg/ml µg/ml (total 10 ml) from 3 liters of THB-grown *S. uberis*.

4. Please amend the paragraph starting on page 31, line 12, as follows.

Cross-reactivity of rabbit anti-SUAM whole protein antibodies and rabbit anti-pepSUAM antibodies between different *Streptococcus* species was investigated. Strains of *S. dysgalactiae* subsp. *dysgalactiae*, *S. agalactiae* (from animals and humans), and *Streptococcus pyogenes* were cultured overnight in Todd Hewitt broth and bacterial surface proteins were extracted in Laemmli sample buffer. SDS-PAGE polyacrylamide gels (7.5%) were electrophoresed followed by transfer to nitrocellulose membranes. They were blocked in PBSTG (phosphate buffered saline, 0.05% (v/v) TWEEN-20, and 0.1% (w/v) porcine gelatin) for 1 h. Affinity purified rabbit anti-pepSUAM and rabbit anti-SUAM antibodies were diluted in PBSTG (1:2000) and blots treated

for 1.5 h. The next treatment after washing blots with several changes of PBST was a 1:2000 dilution in PBSTG of peroxidase-conjugated affipure F (ab') 2 fragment donkey anti-rabbit IgG (H+L). The SUAM protein band was revealed with the peroxidase substrate 4CN (4-chloro-1-naphthol). Western blot results showed cross reaction of pepSUAM and SUAM antibodies with proteins of other *Streptococcus* species, including the human pathogen *S. pyogenes*. The cross reaction with other proteins or protein fragments indicates that SUAM and its functions are conserved or partially conserved between *Streptococcus* *Streptococcus* species and that a vaccine based upon SUAM would have broad application.